04-14-024

FINAL DRAFT

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1/16/2004 11:31:42 AM

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Published as Kentucky Agricultural Experiment Station Article #----- with the approval of the Dean and Director, College of Agriculture and Kentucky Agriculture Experiment Station.

Publication #342 from the Equine Pharmacology, Therapeutics and Toxicology Program of the Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546-0099.

Supported by grants from the USDA Agriculture Research Service Specific Cooperative Agreement #58-6401-2-0025 for Forage-Animal Production Research, the Kentucky Department of Agriculture and the Kentucky Thoroughbred Association Foundation, the Horsemen's Benevolent and Protective Association, and Mrs. John Hay Whitney.

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Abstract

Reviewing the Mare Reproductive Loss Syndrome (MRLS), we proposed that the fundamental mechanism of MRLS, which includes Early Fetal Loss, Late Fetal Loss, Uveitis, Pericarditis and Encephalitis, is tissue penetration by septic barbed setal fragments **(Septic Penetrating Setae, SPS)** from Eastern Tent Caterpillars (ETC). Once ingested, these barbed setal fragments migrate through moving tissues, including blood vessels, followed by rapid hematogenous spread of **Bacteria (B), Bacterial Emboli (BE), and/or Septic Fragments of Setae (Septic Penetrating Setal Emboli, SPSE), together** hereinafter **Septic Materials (SM).** Pathogenic bacteria therefore enter the horse as "hitchhikers" on or in ETC setal fragments, and MRLS is caused by 1) the setal fragments' ability to penetrate moving tissues, including blood vessels, releasing SM, which **rapidly** distribute hematogenously; 2) the high sensitivity of the pregnant mare to bacteria from such SM introduced into the uterus or fetal membranes or fluids, as compared to the non-pregnant horse; 3) the unusually broad spectrum of bacterial pathogens carried on or in the setal fragments, and 4) the less effective antibacterial responses in certain susceptible extracellular fluids, e.g., the fetal, ocular, pericardial and cerebrospinal fluids. The driving force for MRLS pathology, including the abortions, is SM-induced bacterial proliferation, which provides a critical amplification step, enabling 1g caterpillars to **very rapidly** (32 h) cause abortions in 1,500 lb mares. Calculations based on the unique eye data suggest that the actual number of distributing effective SM quanta in field cases may be small, perhaps on the order of 10/horse/day, accounting for the lack of systemic clinical signs in affected horses. In most tissues these small numbers of randomly lodging SM are handled by local antibacterial defenses and yield no clinically significant adverse consequences.

MRLS, therefore, starts with ingestion of caterpillars, followed by barbed setal fragments randomly penetrating intestinal tissues, including thin-walled venules and other blood vessels, with release of SM into blood. SM distribute hematogenously to all points in the body, following cardiac output. The late-term fetoplacental unit represents a large "capture area" for these materials. Although the early-term fetoplacental unit is smaller, both are highly susceptible to insult by SM. The rapid (32 hour) onset of Late Fetal Loss (LFL) in experimental MRLS unequivocally establishes rapid delivery of SM to the fetoplacental unit. A single eye represents the smallest capture area associated with MRLS; the unilateral eye lesions are clinically unique and best explained by hematogenous distribution of discrete infective quanta with penetrating capabilities, such as SPSE. Ocular penetration by SM causes the acute-onset, exudative, treatment-resistant ophthalmitis, followed by loss of vision. The pericarditis cases are also caused by circulating SM, which enter the coronary circulation and migrate to the pericardial space, yielding pericarditis. Bacteria cultured from the pericarditis cases are those associated with MRLS. The pericarditis cases may also be most consistent with and support the SPSE portion of this hypothesis.

Identification of ETC abortigenic activity with the integument of the caterpillar and recent findings of large numbers of granulomatous lesions containing setal fragments in the intestines of ETC-dosed pigs and rats directly supports Step 1, the Septic Penetrating Setal Fragment (SPS) portion of this hypothesis. Analysis of the clinical syndromes and a recently developed toxicokinetic/statistical analysis of MRLS [1] suggest that setally-mediated introduction of SM into blood vessels and other tissues may be key to understanding the toxicokinetics and

pathogenesis of the unique group of syndromes that constitutes MRLS. Like MRLS itself, this hypothesis is unique; the SPSE portion is without precedent, was based on the unique clinical characteristics of MRLS and appears well supported by ongoing experimental approaches.

Background:

Considering MRLS early in July, 2002, we reviewed the well referenced ability of barbed caterpillar setae to penetrate tissues, particularly the human eye [2-4]. It was easy to visualize setal fragments from orally ingested ETC similarly penetrating the intestinal wall, entering small blood vessels, and facilitating entry of bacteria into the blood stream. This simple concept, however, left unanswered how rapid bacterial penetration through distant maternal/fetal membranes might occur, or how the unusual pericarditis lesions or the virtually unique unilateral eye lesions might occur [5,6].

Thinking as pharmacologists, we then considered the consequences if setal fragments or related materials entering blood vessels redistributed hematogenously. If their distribution followed cardiac output, like that of an orally absorbed drug, then the unusual bacteriological and other characteristics of the various discrete clinical syndromes that constitute MRLS, and the lack of clinical signs in affected mares, despite the very rapid onset of experimental MRLS, became more understandable, as we will now detail.

The hypothesis was communicated, on a confidential basis, to selected colleagues. Most were skeptical; one requested detailed explanation. The hypothesis was first formally presented in its Septic Penetrating Setal Emboli (SPSE) format at the 2002 Bain-Fallon Lectures in July, 2002 in Australia, shortly thereafter at the First Workshop on Mare Reproductive Loss Syndrome in Lexington, KY [7] and later registered with the Copyright Office of the Library of Congress. More recently, observations demonstrating the presence of large numbers of setal fragments in the intestinal walls of ETC-dosed pigs [8], rats [9], and a single horse [10] offer strong support for the proposed first step of this hypothesis. Based on this recent experimental evidence consistent with the first step of this hypothesis, and a recent unique toxicokinetic/statistical analysis of MRLS also consistent with this proposed pathogenesis [1], we now communicate a full current draft of this hypothesis, the Septic Penetrating Setal hypothesis of MRLS.

Hypothesis:

We should carefully consider the possibility that simple tissue or intestinal penetration by barbed ETC setal fragments, with associated bacterial "hitchhikers" (Septic Penetrating Setae) followed by hematogenous spread of septic materials (SM) is the fundamental pathophysiological mechanism of each of the five recognized MRLS syndromes: Early Fetal Loss (EFL), Late Fetal Loss (LFL), Unilateral Uveitis, Pericarditis, and the more recently reported Actinobacillus encephalitis.

We now describe this hypothesis in detail and name it the Septic Penetrating Setal hypothesis of MRLS. This hypothesis is specifically written to include penetration of moving tissues by Septic Penetrating Setae, including setal fragments, followed by hematogenous distribution of each or all of bacteria (B) bacterial emboli (BE) or septic penetrating setal emboli

(SPSE), together "Septic Materials" (SM), following initial tissue penetration by septic setal fragments from ETC, which events ultimately yield the differing clinical syndromes comprising MRLS.

1/ Proposed Mechanism:

The pivotal assumption in this hypothesis is that in seeking to understand MRLS, we previously underestimated the combined effects of four key steps:

1.1/ Tissue Penetration--Step 1 is the penetration and migration of barbed caterpillar setal fragments in moving tissues. This hypothesis proposes that exposure to ETC barbed setal fragments results in penetration of the oral and/or intestinal mucosa or other tissues by setal fragments and the nonspecific introduction of local commensal bacteria (bacterial "hitchhikers"), which in the oral cavity may be Actinobacillus and non-hemolytic streptococci. When experimental exposure occurs via the intestinal or other routes, the bacteria introduced may be Serratia spp., a common caterpillar commensal, or other commensal, intestinal or other bacterial "hitchikers" (see Figures 1, 2, 3 and 4).

Figure 1: Eastern Tent Caterpillar. Mature 6th instar Eastern Tent Caterpillar, head to the right, showing distribution of setae. A mature female ETC measures up to 6 cm long and weighs up to 1.5 g, the mature male being somewhat smaller.

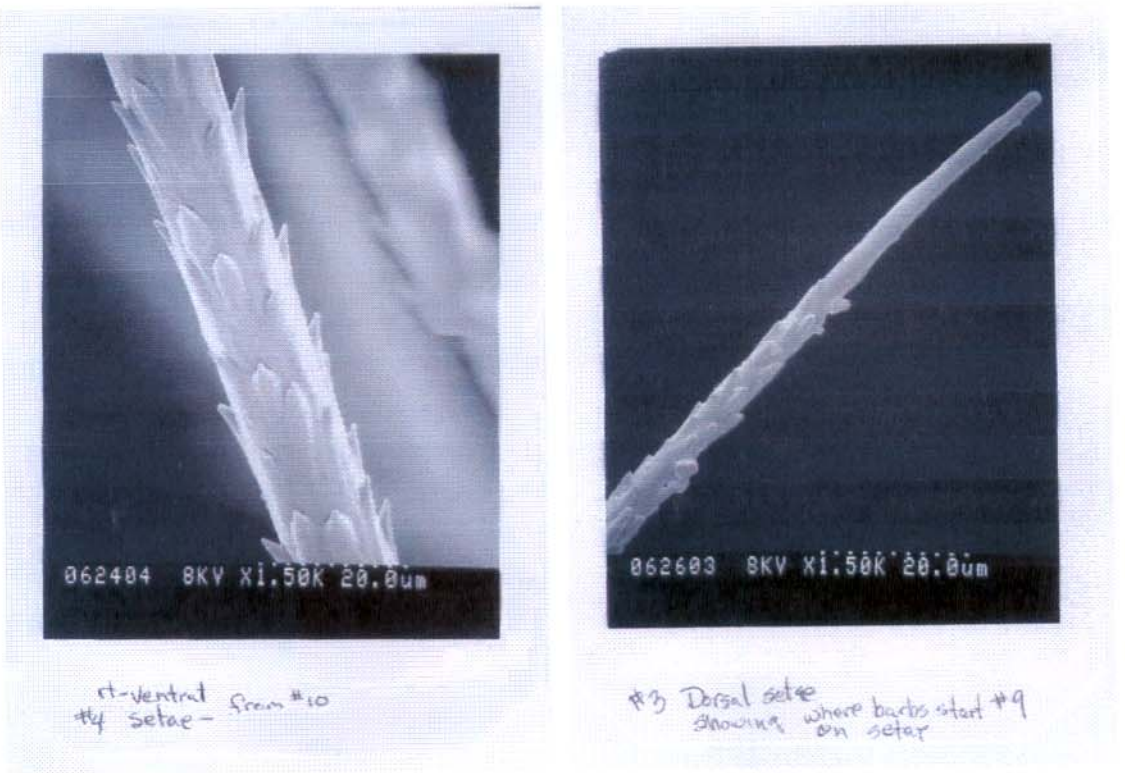

Figure 2: Fine structure of selected Kentucky Eastern Tent Caterpillar setae, showing barbed structures. The left panel shows a portion of the barrel of a seta, approximate diameter 20 mu. Note that the "barbed" structures point towards the tip. The right panel shows the tip of the same setal fragment, again with the "barbs" pointing toward the tip. Note the scale at the base of the photograph, suggesting that the diameter of the setal fragment and its tip is approximately comparable with that of an equine pulmonary capillary. Courtesy of Henry H. Southgate, Research Analyst, Hitachi – 5800 FESEM (Scanning Electron Microscopist); Dr. Ricardo Bessin, Extension Entomologist, University of Kentucky.

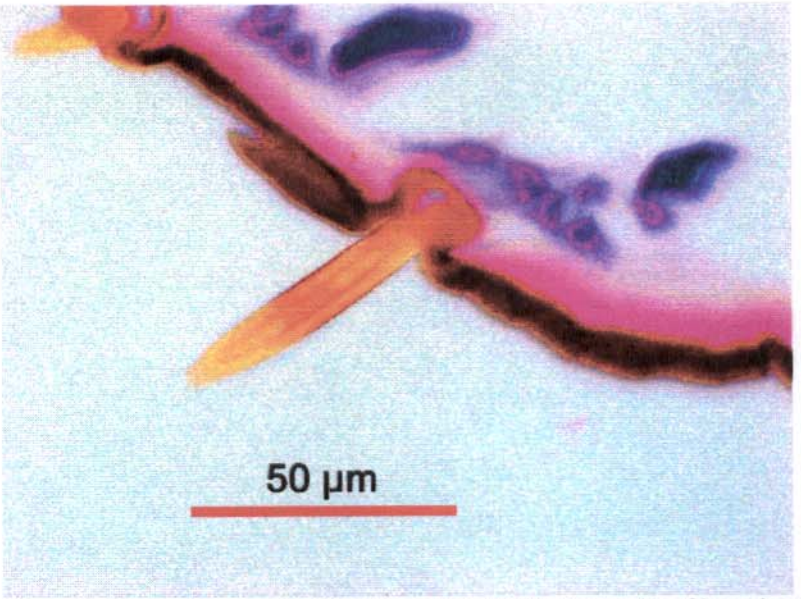

Figure 3: Light micrograph of the base of an ETC setae at site of insertion into the caterpillar integument. Note the possibility that the setal structure is hollow. H&E stain, 400x. Courtesy of Dr. Manu Sebastian, University of Kentucky

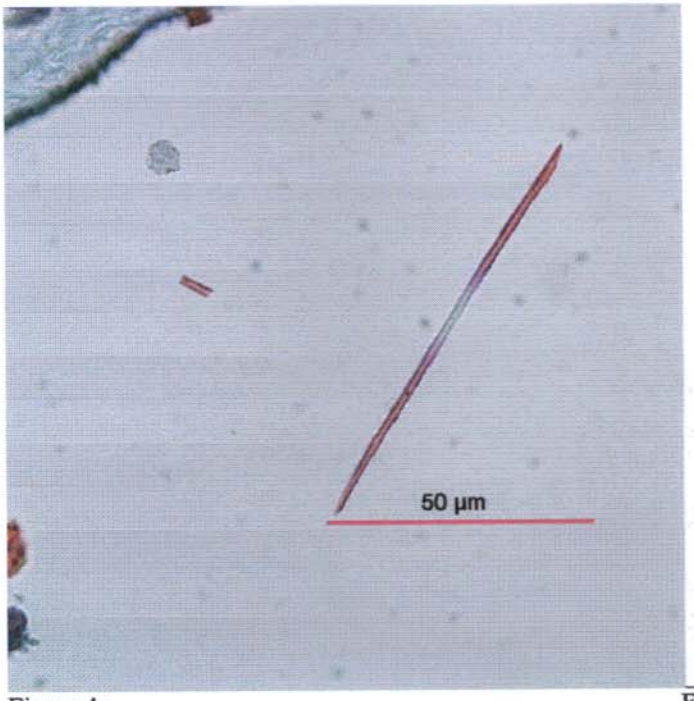

Figure 4a

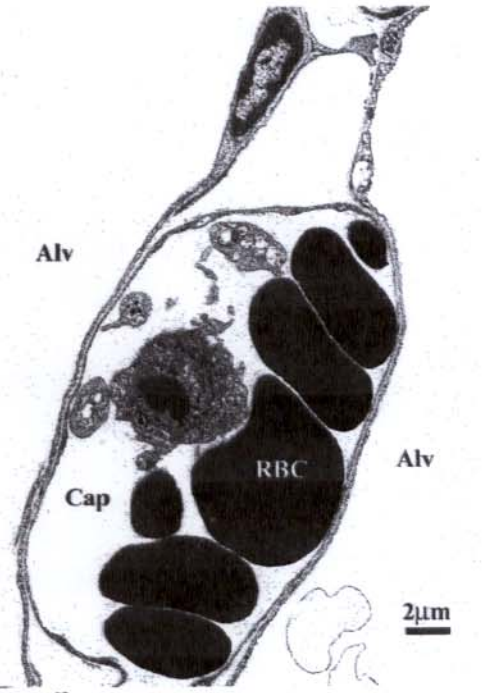

Figure 4b

Fig 4a: Trichrome stained ETC segment at 1000x shows a terminal ETC setal fragment 83 microns in length and 2-3 microns in width, more or less. Caterpillar integument is present in the upper left corner of the photo. 1000x. Courtesy of Dr. John Roberts, University of Kentucky. Figure 4b Electron micrograph of an equine pulmonary capillary with erythrocytes and leucocytes and an apparent . internal diameter approaching 10 mu. Courtesy of Dr. E. Birks, Scott Equine Sports Medicine Building, School of Veterinary Medicine, Kennett Square, University of Pennsylvania.

1.2/ Blood Vessel Penetration and Distribution--Step 2 is the migration of setal fragments, by virtue of their barbed structure, in moving tissues. A proportion of these septic fragments penetrate blood vessels (see Figure 5) and releases SM, which rapidly spread hematogenously in the horse.

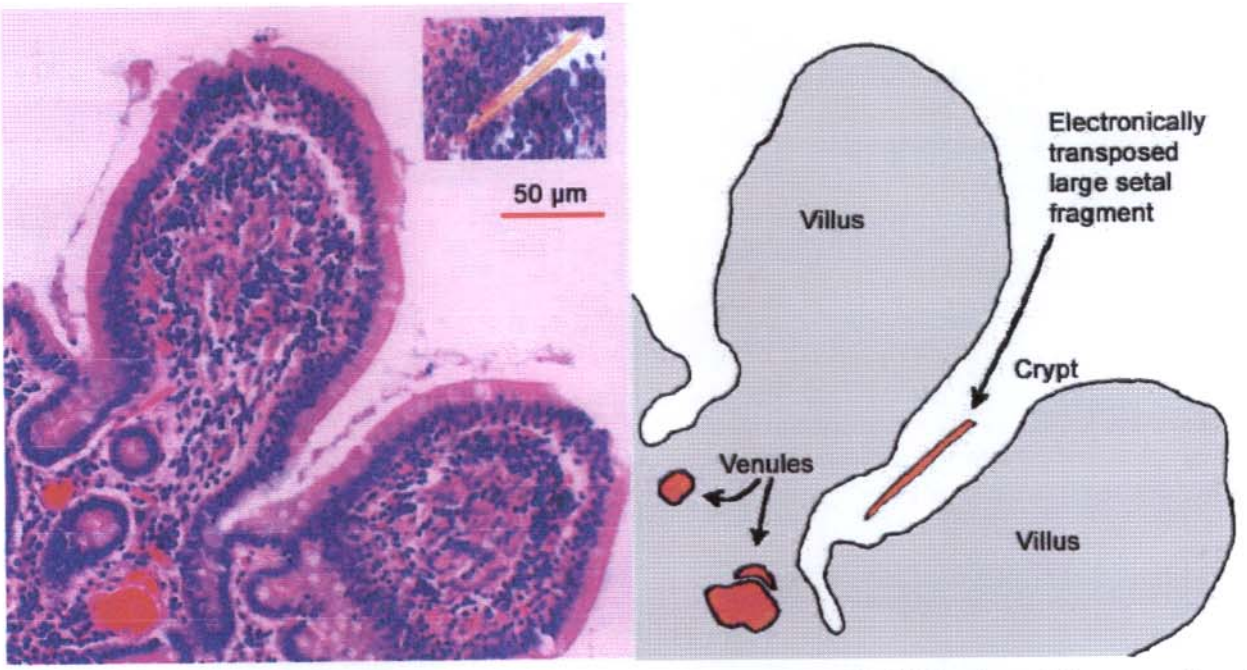

Figure 5: Spatial Relationships with Reference to Intestinal Setal Absorption: The left-hand panel shows a section of mucosa from the small intestine of a horse, presenting, in cross-section, intestinal villi, crypts and numerous thin walled blood vessels, filled with red cells, one vessel about 50 microns in diameter and close to the surface of the crypt. The inset in the left-hand panel shows a large setal fragment found embedded in rat intestine after oral administration of ETC, at the same magnification, electronically transposed. A setal fragment randomly entering the crypt with appropriate barb orientation will move deeper into the crypt driven by intestinal movements, and potentially penetrate the intestinal wall. A fraction of such penetrating setae will randomly enter blood vessels, as indicated in the schematic presentation in the right and panel, and some of which blood vessels will be sufficiently large to accommodate the setal fragment. Note the relatively large size and thin walls of the large blood vessel outline in the schematic presentation, and the relatively short distance between the intestinal exterior at base of the crypt and the blood vessel itself. Courtesy of Dr. Manu Sebastian and Mr. Charlie Hughes, University of Kentucky.

1.3/ Distant Tissue Penetration--Step 3 is the hematogenous relocation of SM to points distant from the point of entry, their retained ability to penetrate moving tissues, and the apparently high sensitivity of the fetoplacental unit to SM, especially as compared with the sensitivity of the non-pregnant horse and with mice, rats, and possibly other animals.

1.4/ Variable Defense Resources--Step 4 is the poor anti-bacterial responses in clinically affected tissues. Rapid bacterial growth follows bacterial contamination of fetal and other extracellular fluids, which results in fetal loss [11]. The eye is immunologically privileged, and its extracellular fluids are less well protected than many tissues [12]. The brain and cerebrospinal fluid also have immunological deficits, as highlighted by Equine Protozoal Myelitis (EPM). Alone among the clinically affected tissues, a clear immunological deficit cannot be associated with the pericarditis lesions, although the pericardial fluid itself may, as an extracellular fluid, be less well protected. Additionally, it may be that the pericardial lesions relate in part to the motility and

central location of the heart in the circulatory system and its resultant high level of exposure to blood-borne SM, especially SPSE.

In most tissues, small numbers of "lodged" SM are readily handled by the specific and non-specific immune systems and cause no clinically significant or apparent long-term damage. However, the lack of effective anti-bacterial systems in fetal fluids appears to leave the fetus largely unprotected once bacterial contamination of fetal fluids occurs, leading rapidly to the EFL and LFL manifestations of MRLS (Table 1). The nonspecific nature of the proposed bacterial transport system is consistent with the wide range of bacterial pathogens recovered from MRLS fetuses (Table 1).

Like MRLS itself, this hypothesis is unique, and the Septic Penetrating Setal Emboli portion of this hypothesis is apparently without precedent in biology or medicine. In its support, this hypothesis well fits the unique toxicokinetics of MRLS set forth in an earlier communication[1] and also well fits the unique grouping of clinical syndromes that constitutes MRLS. Additionally, the hypothesis is also well supported by recently acquired evidence concerning multiple setal fragment penetrations in the intestines of ETC-dosed pigs, rats and horses (see Figures 6, 7, and 8), the unusual speed of onset of the abortions, calculations concerning the likely numbers of circulating SM quanta, the difficulty in culturing bacterial pathogens from the blood of affected horses and the unusual general lack of systemic clinical signs in MRLS affected horses.

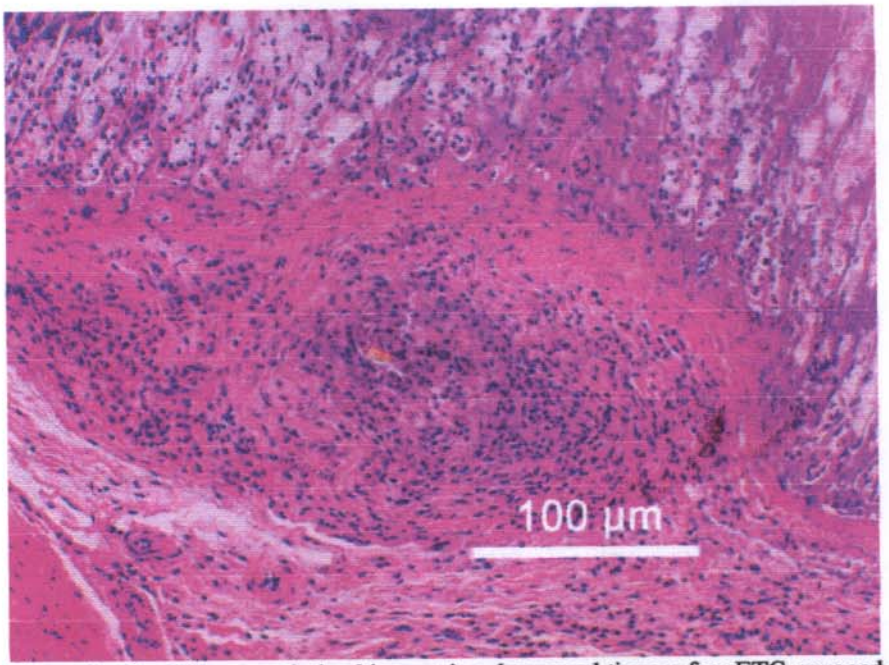

Figure 6a: Setal fragment lodged in gastric submucosal tissue of an ETC-gavaged rat, surrounded by an extensive granulomatous reaction. Courtesy of Dr. Terrence Fitzgerald, State University of New York, Cortland, and Dr. Manu Sebastian, University of Kentucky.

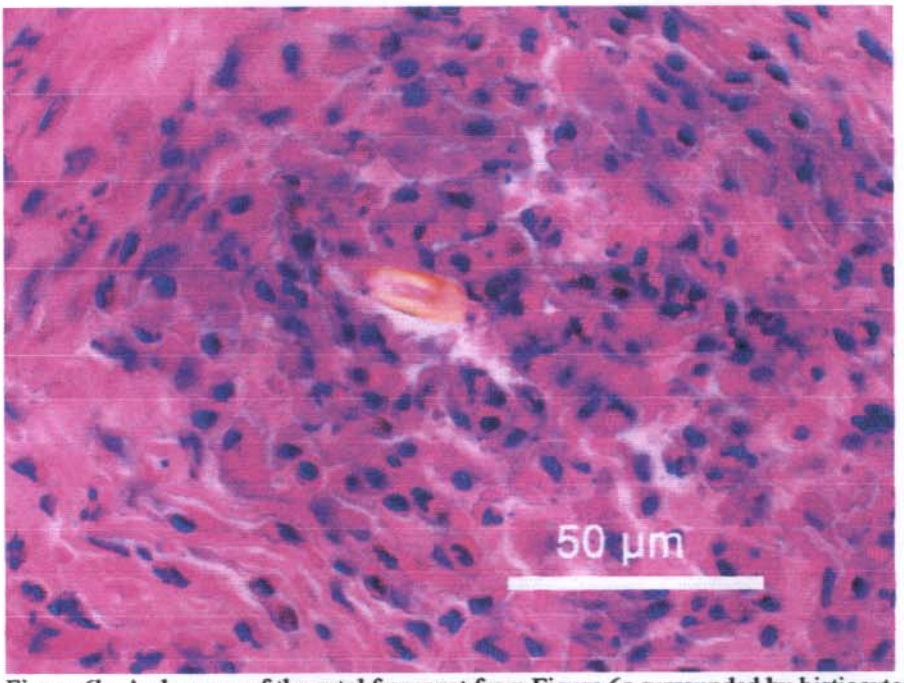

Figure 6b: A close-up of the setal fragment from Figure 6a surrounded by histiocytes, H&E stain, 400x. Courtesy of Dr. Terrence Fitzgerald, State University of New York, Cortland, NY and Dr. Manu Sebastian, University of Kentucky.

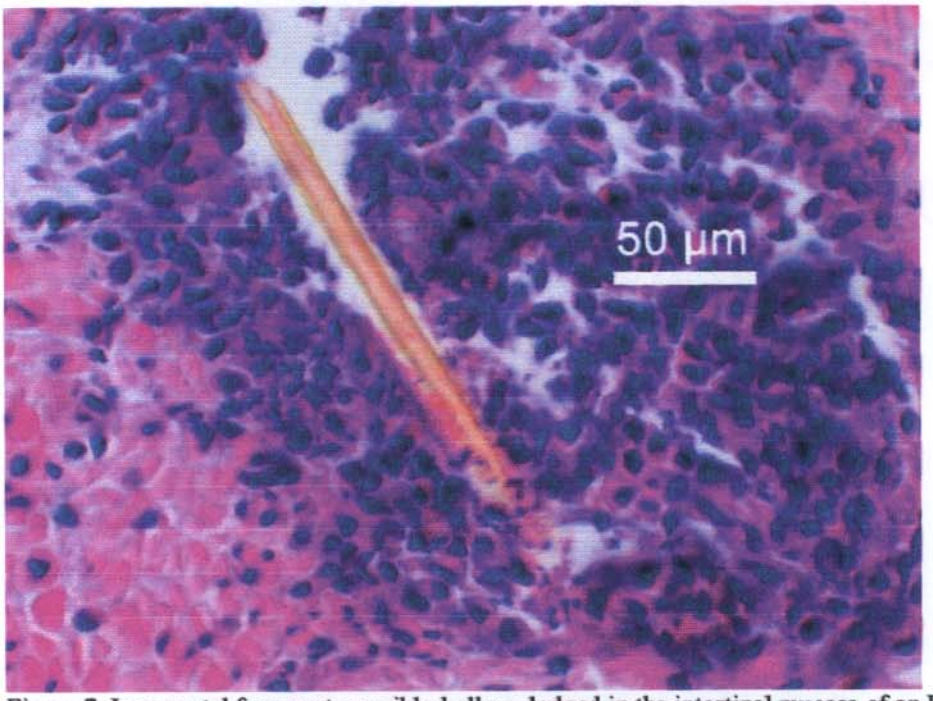

Figure 7: Large setal fragment, possibly hollow, lodged in the intestinal mucosa of an ETC-gavaged rat, surrounded by inflammatory cells and granulomatous reaction. H&E stain, 400x.

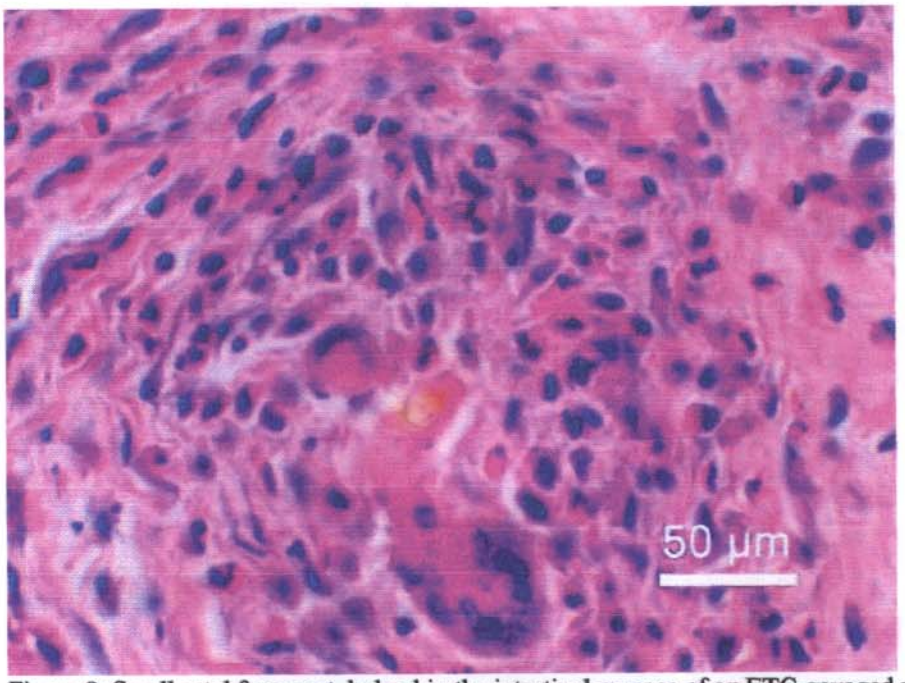

Figure 8: Small setal fragment, lodged in the intestinal mucosa of an ETC-gavaged rat, surrounded by inflammatory cells, including a multinucleated giant cell, H&E, 400x. Courtesy of Dr. Terrence Fitzgerald, State University of New York at Cortland, Cortland, NY, and Dr. Manu Sebastian, University of Kentucky.

2/ HEMATOGENOUS SPREAD OF SM AND MRLS SYNDROMES:

2.1/ From clinical cases and experimental work, it appears that the initiating event in each of the MRLS-associated syndromes is local bacterial infection of hematogenous origin, with the bacteria initially entering the blood stream at the site of exposure to the caterpillar.

2.2/ The cardiac and encephalitic syndromes cannot be other than hematogenous in origin.

2.3/ The unilateral ophthalmitis cases are also consistent with and best explained by a hematogenous source. In particular, a number of these cases apparently started with hemorrhage deep in the eye, and the difficulty in treating these cases is also consistent with a septic hematogenous insult originating deep in the eye. Additionally, none of the affected eyes was fluorescein-positive, suggesting that no significant corneal damage or penetration had occurred. Beyond this, recent experimental work has shown that topical exposure to caterpillars fails to reproduce characteristic MRLS-associated eye lesions[a], further suggesting a blood-borne ophthalmitis.

3/ PRELIMINARY SUPPORTING EXPERIMENTAL EVIDENCE:

3.1/ Analysis of our LFL experimental work in 2002 [13] suggested to us that bacterial proliferation was the driving force in LFL, and that the source of the fetal bacterial pathogens was hematogenous and **not** ascending (i.e., via the cervix) in origin. This observation was key in

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[a] Personal Communication, Dr. Bill Bernard, Rood and Riddle Equine Hospital, Lexington, KY.

developing this hypothesis. Previously, it was considered that a *toxin* killed the fetus and that the bacteria cultured from the fetus were secondary invaders.

3.2/ This hypothesis was next supported by experiments in 2002 by Bernard and co-workers in which EFL was reproduced in mares administered ETC integuments by stomach tube [14]. Similarly, 2002 experiments in pregnant mice suggest that intraperitoneal administration of a homogenate of fresh setae may lead to re-absorption of fetuses in three of six treated mice [15], although this experiment did not repeat in one attempt with frozen ETC setae.

4/ CLINICAL CONSEQUENCES OF BARBED FOREIGN BODY MIGRATION:

4.1/ Background:

In this section we review, in summary form, the literature on the clinical, microbiological and pathological changes associated with penetration by barbed foreign bodies. The biomedical literature on barbed structures includes those associated with tarantulas, porcupines, stingrays, the Portuguese man-of-war, cacti, the Phoenix Date Palm and the pappus plant of South Africa. This review leads to some general conclusions concerning the pathophysiology of conditions caused by or associated with barbed structures.

4.2/ Caterpillar Setae:

Caterpillar setae are often barbed and have been recognized to penetrate the human eye. Since the 19th century, caterpillars have been reported to cause a condition known as ophthalmia nodosa, where a granulomatous nodule formed on the conjunctiva and iris in response to caterpillar setae [16]. There have been numerous reports since of endophthalmitis, conjunctivitis, and uveitis caused by direct ocular exposure to caterpillars [2-4,17] or following dissemination of setae by wind [18] and these literature reports formed an important starting point for this setal hypothesis of MRLS.

Although setal penetration of tissues other than the eye is rarely described in the medical literature, one retrospective study of ten patients presented to a pediatric emergency department following ingestion of caterpillars detailed the extent of setal lesions not associated with the eye [19]. Caterpillar setae were removed from the tongue (n=7), lips (n=5), oropharynx (n=5), cheeks (n=4), buccal mucosa (n=4), abdomen and legs (n=2), esophagus (n=1), and neck (n=1). The lesions had no long-term effects, and none of the patients experienced anaphylactic reactions.

Caterpillar setae have caused numerous cases of dermatitis through direct and airborne contact with human skin. Because vacationers annually developed an erythematous dermatitis including vesicular eruptions while camping in the dunes region of Ameland, Netherlands, an experimental study was performed to assess if the caterpillars of brown-tail moths (*Euproctis chrysorrhoes* Linn.) were the cause. Setae from the caterpillars were taped to the backs of volunteers, and dermatitis occurred in over 70% of the volunteers [20].

One hundred sixty-five soldiers and civilians from the US military community in Heidelberg, Germany required treatment for acute dermatitis during one week in the summer of

1995, which was 144 more cases of dermatitis than the average of 3 per day. Those affected complained of a painful, itching rash distributed widely over the body. Urticarial hairs from the oak processionary caterpillars, which live in communal webs on oak trees, were identified as the cause. Review of medical cases in the area revealed that outbreaks of dermatitis associated with the oak processionary caterpillar were reported throughout that region in 1995 [21].

Other species of caterpillars have been reported to injure large numbers of military personnel. Urticaria from dead caterpillars and old cocoons of *Thaumetopoea wilkinsoni* Tams caused a rash and severe irritation to 600 soldiers camped in a pine grove in Italy [22]. Furthermore, pine processionary caterpillars (*Thaumetopoea pityocampa* Den. & Schiff.) are reported to often cause dermatitis among military personnel and dependents in Livorno, Italy [21].

In Sydney, Australia, several employees and clients at a community center experienced skin reactions and irritations for over 4 months. Outside the center, a large colony of caterpillars was living in a eucalyptus tree about 10 meters from the intake vent of the air-conditioning system. Caterpillar-like hairs were identified in samples of dust from inside the center. Based on reports of successful use of sticky tape to sample affected areas for jellyfish stings, caterpillar hairs were removed from two of the three people thus tested. Following removal of the caterpillar nest and thorough cleaning of the center, no further dermatitis cases were reported [23].

4.2/ Other Barbed Structures:

We have reviewed the literature on pathological changes associated with tissue penetration by barbed structures from tarantulas [24], porcupine quills [25], stingray barbs [26,27], the Portuguese Man-of-war [28], catfish [29], cacti and the Phoenix Date Palm [30,31], the pappus plant of South Africa [32] and grass awns [33]. These varied clinical reports show that barbs are a widespread means of protection/aggression in nature. Barbed structures may enter the body by any route, including the intestine. Once lodged, barbed structures migrate, the rate of migration depending only on the rate of movement of the host tissue. Tissue movements serve to "ratchet" the barbed structure along in entirely random directions through any moving tissue. Most of the barbed structures noted above are relatively large and take days, weeks or even months to migrate through soft tissues to the point of clinical presentation.

To summarize, review of the literature suggests that the clinical consequences associated with tissue penetration by barbed fragments are always local and directly associated with the physical presence of the barbed structure. Second, barbed fragments migrate relatively slowly through most tissues, although given sufficient time, usually weeks or more, they can penetrate anywhere, including into the brain through the foramen magnum. However, exposure to ETC setal fragments can cause abortions in pregnant mares within hours of their intubation.

To maximize the effectiveness of barbed structures as a defensive mechanism, the numbers of barbed structures deployed should be large. Additionally, a means of accelerating their distribution through the target body would be helpful. We propose that the barbed setal fragments of ETC have effectively solved these problems. The setae are present on ETC in large numbers, and fragmentation presumably further increases that number. The fragments, or some portions of the fragments, however, are sufficiently small that at least a fraction of them

distribute hematogenously and, therefore, rapidly as SM, SPSE or equivalent materials, giving rise in the horse to the five distinct syndromes that constitute MRLS, and whose onset, both in the laboratory and in the field can, for LFL at least, be extremely rapid.

5/ BACTERIA/BACTERIAL EMBOLI/ SEPTIC PENETRATING SETAL EMBOLI:

5.1/ Septic Penetration:

The second step in this hypothesis is that the setal fragments penetrate blood vessels, especially thin-walled veins, and release SM, a proportion of which move rapidly in the blood to new and more distant locations in the body. All such SM movements are passive, secondary to tissue movement and/or blood flow (cardiac output), and all events are statistically determined. These events presumably occur at some level in all species exposed to ETC, apparently with only occasional adverse health consequences, as indicated by the relatively low incidence of adverse events in non-pregnant horses and other field exposed and experimentally exposed species.

5.2/ Starting Numbers of Septic Penetrating Fragments:

For this mechanism of MRLS to be operative, and especially for the SPSE portion of this hypothesis to be effective, the starting number of setal fragments should optimally be large, since all penetration and distribution events are statistically determined. This requirement is not inconsistent with our recent estimates suggesting that aborting mares were exposed to the equivalent of 5 to 30 g/day of ETC [1]. Beyond this, recent reports that the intestinal tracts of pigs necropsied following exposure to broadly equivalent doses of orally administered ETC contain very large numbers (estimated 10^5 or 10^6 per pig)[9] of small setal fragments encased in microgranulomatous masses are entirely consistent with the Septic Penetrating Setal portion of this hypothesis.

5.3/ The Septic Materials (SM) Distributed:

This hypothesis is specifically written to cover three distinct possibilities. It assumes the primary entry of the pathogenic bacteria is associated with the penetrating setal fragments. The second step, hematogenous spread of SM, may involve distribution of either B, BE, or SPSE. While some of the clinical characteristics of MRLS may best be explained by the SPSE hypothesis, the possibilities that the SM are simply B or BE distributed by blood flow are specifically included in this hypothesis. In any event, distribution and entry of the SM into the fetal membranes occur, and apparently the pregnant mare is highly susceptible to both Early and Late Fetal Loss from hematogenous exposure to the SM.

5.4/ Distributing SPSE and the Lung:

The SPSE portion of this hypothesis requires that hematogenously spread setal fragments either pass through or bypass the lung capillary beds to enter the systemic circulation. While the ETC setal fragments observed in pig intestinal tissues are small barbed cylinders of about 15-25 microns in diameter and 100 microns in length, the question arises as to how such fragments

could pass through the lung capillary beds. We suggest three mechanisms that may allow movement of SPSE past or through the lung capillary beds and into the systemic circulation.

An obvious mechanism is that SPSE move through the lungs in the same way as they enter into and move through the intestine, namely that they are driven or "ratcheted" along through lung tissues by respiratory movements, which will, sooner or later, allow passage of a fraction of SPSE entering the lung.

SPSE may also bypass lung capillary beds through well-characterized anatomical and pathological shunts. Studies by Gillespie [34] and Littlejohn and others estimate the percent venous admixture (anatomical shunts) in the pulmonary circulation to be about 5% in normal horses, but 9% in horses afflicted with emphysema, which are pathological shunts [35]. In another study of respiratory function, the percent venous admixture (shunted fraction) was determined to be about 9% in normal horses and 26% in horses with Chronic Obstructive Pulmonary Disease (COPD). Anatomical and pathological shunts therefore provide a mechanism for SPSE to rapidly bypass lung capillary beds and enter the systemic circulation.

Finally, it may also be that some SM/SPSE fragments are themselves small enough to pass relatively unhindered through capillary beds, as suggested by the small diameter of some terminal setal fragments (Figures 2b and 4) compared with the diameter of equine lung capillaries.

6/ GENERATING BACTEREMIA, BACTERIAL EMBOLI OR SEPTIC PENETRATING SETAL EMBOLI: THE PROTECTIVE FUNCTION OF SETAE FOR THE CATERPILLAR:

6.1/ Clinical Evidence for Tissue Penetration by Caterpillar Setae and Human and Other Hairs:

Historically, the ability of "hairy" caterpillars to cause oral lesions in horses is specifically noted in an un-referenced citation in the sixth edition of Blood and Henderson [36] which states *"Elongated shallow erosions (2 x 0.5 cm) can occur <u>in profusion</u> in the mouths of a large proportion of a band of horses grazing pasture infested with hairy caterpillars or pastures containing grass with bristly seedheads"* (our underlining). Beyond this, our fundamental assumption that small barbed setal fragments can penetrate moving tissues will come as no surprise whatsoever to members of the hairdressing and dog grooming professions, where the ability of short hair fragments to penetrate human skin, usually in the spaces between the fingers or toes (note also Figure 5) and cause cutaneous conditions known as trichogranulomas and pilonidal sinus is a widely recognized hazard in these professions[b] [37,38].

6.2/ The Role of Intestinal Absorption:

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[b] First communicated to TT by an equestrian colleague, Nancy DeJarnette, Bennet Williams Hair Salon, Lexington Kentucky, 2002, thereafter rapidly confirmed by a literature search.

For the purposes of this hypothesis, we may view the equine intestinal tract, with its crypts and villi and ongoing peristaltic movements, as an ideal organ to "trap" barbed setal fragments, propel setal fragments into and through its tissues and, with its network of apparently thin walled absorptive blood vessels, as a body system highly likely to yield B, BE or SPSE following ETC exposure (Fig 5). The findings that Serratia are associated with both ETC and MRLS and that Actinobacillus and non-hemolytic streptococci may be oral commensals also focus attention on the introduction of ETC surface bacteria and/or oral commensal bacterial into the blood stream as an integral part of the pathogenesis of MRLS. In this regard, the apparent efficacy of muzzling mares during the 2002 caterpillar season seems to suggests considerable importance for the oral route of exposure [7].

7/ THE STRUCTURE AND FUNCTION OF CATERPILLAR SETAE:

To our knowledge, little attention has been given to the fine structure and function of simple barbed setae in ETC and similar caterpillars. These structures have presumably evolved in a manner calculated to maximize the digestive discomfort and/or other adverse responses associated with their ingestion, it not being in the interests of ETC to be perceived as palatable. While the setae of ETC are readily distinguishable from poisonous setae, they presumably also serve a protective function for the caterpillar.

We should therefore consider the possibility that mechanical penetration and facilitation of bacterial entry is an aversive/defensive mechanism associated with barbed setae of ETC and other caterpillars. In this regard, these setae may well have evolved so that their setal protective mechanism is to facilitate the entry of bacterial pathogens into caterpillar predators. The introduced bacteria then proliferate and serve a destructive function broadly similar to the enzymatic toxins of the poisonous Lonomia caterpillar species [39]. This is, presumably, a cost-effective defensive strategy for ETC and related caterpillars, the bacterial pathogens substituting for the poisons, toxins or venoms of other species, at a presumably significant saving of resources for the caterpillar.

This setal defense mechanism may work best when the dose to the affected animal is relatively high, as it would be on a caterpillar-to-bird weight basis, rather than the much smaller caterpillar-to-horse ratio operative in MRLS. Furthermore, this is presumably an ancient caterpillar defense mechanism; as such it would also not be surprising if setal fragments had specific factors or materials associated with them to facilitate bacterial transportation or tissue penetration, much as the saliva of blood sucking parasites contains specific inhibitors of blood coagulation; in fact, loss of such associated facilitating factors may be a limiting for ETC abortigenic activity. Finally, the familiar clustering behavior of ETC may suggest a classic cooperative defensive mechanism at work, with the clustered group minimizing their individual risk/exposure, while and the same time maximizing their group setal defense (see Figure 9).

Figure 9: A cluster of mature Eastern Tent Caterpillars, on their tent, showing group clustering behavior and fine setal structure. Courtesy of Dr. Terrence Fitzgerald, State University of New York at Cortland.

There may also be dimensional considerations in the overall process of MRLS. The large size of the horse presumably means a similarly large number of intestinal venules with dimensions appropriate for setal capture and initiation of the hematogenous redistribution events associated with the SM, and especially the SPSE, portions of this hypothesis, as appears to be the case in Figure 5. In this regard, it is of interest to note that attempts to reproduce MRLS in pregnant rats and mice have not been particularly successful, while experiments in swine have apparently been more productive.

MRLS, as we know it, may therefore simply be a recent per-acute manifestation of a long established ETC defensive mechanism. It was on this basis that, as soon as ETC became available to us in 2002, we directed our attention to their setae. For reasons not readily apparent, the pregnant mare is exceptionally sensitive to this defense mechanism. Non-pregnant horses, however, show a small level of clinical response, as indicated by the very low incidence of eye and heart syndromes in non-pregnant horses from the 2001 and 2002 MRLS data.

8/ UNUSUAL SUSCEPTIBILITY OF THE PREGNANT MARE TO SM:

8.1/ The Role of Myometrial and General Locomotor Activity:

B, BE or especially SPSE lodged in a uterine blood vessel of a pregnant mare will again migrate through these tissues when the mare moves. Myometrial movement, either due to the musculature of the myometrium itself or the physical activity of both the mare and fetus, will drive migration of B, BE, or a lodged SPSE. Eventually, the SM will penetrate the fetoplacental unit.

8.2/ The Role of Fetal Membrane Penetration:

Well established clinical experience suggests that very modest bacterial contamination of amniotic fluid can result in rapid bacterial overgrowth, followed by death and expulsion of the fetus within a day or days[c]. Based on this experience, penetration of the fetal membranes by a modest quantity of SM would presumably be sufficient to produce EFL or LFL. Review of our 2002 ETC/LFL experimental data [13] suggested to us that bacterial proliferation was a primary or driving event in LFL, apparently occurring prior to signs of fetal distress and fetal death. As mentioned earlier, identification of the key driving role of bacterial proliferation in the LFL syndrome was a critical factor in the development of this setal hypothesis of MRLS.

8.3/ The Role of Fetal Size and Movement:

A striking characteristic of experimental MRLS is the speed with which experimental LFL can occur, with recent (2003) work showing that the first LFL losses occurred within 32 hours [1]. We suggest that the late fetus presents a large "capture area" for randomly distributing SM; as such, a late fetus is statistically more likely to be "hit" by randomly distributing SM within a given period than a much smaller early fetus. Additionally, uterine movements are greater in a mare carrying a late fetus, driving the tissue migration of SM lodged in uterine tissue, and ensuring their rapid penetration through a fetal membrane. Together, these steps may immediately explain the apparently more rapid onset of LFL than EFL, and especially the extremely rapid onset of high dose experimental LFL.

8.4/ Role of Delayed Fetal Membrane Penetration:

This hypothesis also readily explains cases of EFL or LFL occurring at some time after exposure to caterpillars has ceased. Inopportune location of SM, especially SPSE in a less mobile area of the myometrium will delay the entry of SM into the fetal membranes, yielding EFL or LFL at some time after exposure to ETC has ceased.

8.5/ The Critical Lack of Positive Blood Cultures and Systemic Clinical Signs:

The SPSE and to some extent the BE models are consistent with the lack of positive blood cultures and virtual lack of systemic clinical signs of bacteremia from EFL and LFL mares. This is because the blood-borne bacterial contamination is carried in small numbers of discrete quantal packets on or in individual setal fragments or bacterial emboli and not diffused throughout the bloodstream. The fact that it has been difficult to identify positive blood cultures in MRLS mares may be more consistent with the SPSE portions of this hypothesis than with the simple B or BE portions.

Similarly, the lack of clinical symptoms in mares undergoing LFL within hours of ETC intubation speaks majorly against simple setally induced "loss of intestinal integrity" and resultant bacteremia (B) inducing LFL. In our hands [40,41], IV infusion of small amounts of lipopolysaccharide endotoxin (LPS) is more than sufficient to rapidly induce significant and easily discernible systemic clinical signs and clinical chemistry changes in horses. Given the

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[c] Dr. Jim Bowen, Blacksburg, Virginia, Personal Communication, July 2002.

sensitivity of horses to small amounts of LPS, it is difficult for us to imagine a sequence of events in which a loss of intestinal integrity sufficient to induce abortions with 32 hours would occur without detectable clinical or clinical chemistry changes in the mare(s) in question.

8.6/ The Need for an "Amplification Factor":

One of the most striking aspects of MRLS is the apparently enormous potency of the caterpillar "factor" on a body weight basis. Caterpillars are very small (about 1 g) compared with horses; our experience in toxicology suggested the improbability of small numbers of caterpillars themselves producing sufficient quantities of any orally absorbed toxin to cause abortions in 1,500 lb mares, let alone a toxin with essentially no other current or previously recorded signs of toxicity. This question has led to suggestions of a required biological amplification step (viral contaminants, fungal overgrowth on frass, etc) to provide the requisite amplification factor

The bacterial proliferation step described herein itself provides the requisite biological amplification/ multiplication factor, presumably enabling a single septic penetration of a fetal membrane to produce EFL or LFL in a 1,500 lb mare.

9/ UVEITIS, PERICARDITIS AND ENCEPHALITIS CASES:

This hypothesis requires that all horses in central Kentucky, and not just pregnant mares, exposed to ETC suffer essentially equivalent episodes of SM spread. We propose that the uveitis, pericarditis, and encephalitis cases, which occurred across central Kentucky in horses of both sexes and all ages, are clear evidence of this process at work in a systematic fashion, albeit at a very low level (1/1,000 or so), in the entire "at risk" population of horses during the period when MRLS occurred in central Kentucky during 2001.

9.1/ The Unilateral Uveitis Cases:

We consider it well established that the cases of uveitis are of hematogenous origin. We propose that the primary hematogenous event is delivery of a quantum of SM to the eye. The very low incidence of uveitis observed is presumably related to the relatively small target size ("capture area") and the equivalently small fraction of cardiac output supplying an individual eye.

A significant argument in favor of the SPSE portion of this hypothesis is the fact that all incidents of uveitis were unilateral. This observation well fits a quantal and entirely random hematogenous distribution of SPSE, as compared with hematogenous distribution of BE, and especially B, delivery of which are less likely to be so clearly quantal in nature.

With reference to Step 4 (section 1.4 above), the eye is also an immunologically privileged area; as such, it may be particularly susceptible to damage by penetrating SM. Our ability to observe eye pathology associated with MRLS is also most likely due to the ease of observing events occurring in the eye, and the highly significant consequences of eye damage compared with limited local damage in other areas of the body, and the possible therapeutic and immunological difficulty of controlling a septic focus that has entered deep in the eye.

The small number of unique unilateral uveitis cases may allow us to define a lower limit for the total number of "effective SM quanta" that distributed in the blood of the average horse in central Kentucky during MRLS 2001. If we assume about 30 cases of unilateral uveitis in about 30,000 exposed horses, we have an overall minimum "effective SM quantal hit" rate, (at 2 eyes per horse), of about 60,000/30 or 1/2,000 eyes. Based on these figures, the probability of any single horse in central Kentucky showing two "overlapping" cases of "unilateral" uveitis is not less than about 1 in 4,000,000.

Assuming that each eye weighs about 0.25lb, then overall average 2001 "hit probability" is 1per 500 lb of equine tissue, or about 2 total SM quanta per horse for the entire 2001 MRLS period. A mature fetoplacental unit, which weighs about 140 lbs, will have, on a mass basis, about a 25% probability of a single effective "hit". Similarly, the early fetoplacental unit, which weighs in at about 25lbs, should expect an overall hit rate in the area of about 2-3%. While these figures bracket the overall EFL/LFL rate of about 17%, they do not appear to reflect the apparently much greater rate of EFL during MRLS 2001. However, it should also be noted that these estimated total average numbers of distributing effective SM quanta are extremely small, much less than one effective SM quanta per horse per day.

These estimates are, of course, averages calculated over the entire population of horses at risk in the 2001 season in central Kentucky. Individual farms, individual groups of horses and especially individual horses will have had much greater rates of exposure; however the take home message from the eye data and these calculations is that the actual numbers of effective SM quanta actually reaching the systemic circulation is likely to be quite small, even during an acute epidemic of MRLS.

9.2/ The Pericarditis Cases:

The pericarditis cases presumably represent SM that enter the coronary blood supply, lodge in blood vessels, and then migrate through the moving/contracting cardiac vessels and tissue. Of all tissues in the body, the contracting heart is one through which one might expect B, BE, or especially SPSE to migrate fastest. Additionally, for every B, BE or SPSE that migrated out and appeared at the epicardial surface, at least one migrated in the opposite (or other) directions. Presumably, a larger number of sub-clinical epicarditis cases occurred associated with the MRLS episodes, and resolved spontaneously, as presumably do most incidents of systemic SM spread in non-pregnant horses.

A problem with this portion of the hypothesis is that pathologists have, to date, reported no evidence of setal tracks in cardiac tissues. A careful search should be made for signs of sub-clinical pericarditis associated with intestinal exposure to ETC. The central role of the heart in the circulatory system and its ongoing contractile activity may suggest a considerable probability of transient positive histological and bacteriological culture findings in pericardial fluid associated with ETC exposure. On the other hand, analysis of the recent pig data suggests that the penetrating setal fragments are relatively small, and the eye data suggests that the actual numbers distributing may be very small, with numbers in the order of 10-100 per horse, representing a relatively large number of distributing SM quanta. Given this circumstance, it may

be challenging to visually detect setal tracks in cardiac tissue. In this regard, it is worth noting that the microgranulomas associated with lodged setae in the intestinal tract of pigs and rats are themselves very small, and not grossly observable [9].

Bacteria cultured from the pericarditis cases are those associated with MRLS, but no bacteria were cultured from some pericarditis cases, which may suggest loss of septic contaminants during passage of the SM through cardiac musculature. The pericarditis cases may also be most consistent with and best explained by the SPSE portion of this hypothesis.

9.3/ The Encephalitis Cases:

Since MRLS was first described, three specific cases of Actinobacillus encephalitis have been recognized as occurring in the same period. Like the pericarditis cases, these cases are unquestionably hematogenous in origin and occurred in about the same time period as MRLS [13].

10/ INTELLECTUAL ECONOMY OF THE HYPOTHESIS:

10.1/ We early focused on ETC setae because of their well established overall role in caterpillar defense mechanisms. This hypothesis is a modification and simplification of the hypothesis that drove our first mouse setal experiment, which assumed that the setae were introducing a protein or other toxin or factor that was the primary pathogen. Further reflection, along with identification of bacterial proliferation as the driving force in MRLS, suggested that a setal toxin is not necessarily required, as set forth above, and the speed of onset of LFL in laboratory experiments also does not suggest a classic catalytic toxic mechanism. For example, the enzymatic fibrinolysin toxin of the Lonomia caterpillar in Brazil takes up to one week to kill a human, consistent with an ongoing low-level catalytic/toxic activity [42]. In contrast, experimental LFL abortions can occur within 32 hours of exposure to ETC.

10.2/ This hypothesis does not require the presence of any extra toxins in the ETC, or viruses, or Microsporidia, or unusual weather patterns, cyanide precursors or cyanide, or plant toxins, or frass, or fungal overgrowth on frass; it simply requires ETC.

10.3/ We must note that a clear characteristic of MRLS has been that no evidence of toxins, viruses, mycotoxins, etc, other than the bacterial species set forth in Table 1, have thus far been associated with this syndrome, despite extensive searches for the same. In fact, the only equine micropathology directly linked to the caterpillars themselves are the recently recognized intestinal granulomatous lesions associated with ETC setal fragments [8,9].

10.4/ This hypothesis may also explain why no significant hormonal patterns or other clinical chemistry changes have yet been identified in the aborting mares. EFL and LFL are dependent on the direct and relatively nonspecific seeding of small amounts of bacterial contaminants into the fetal membranes, followed by bacterial proliferation and abortion.

10.5/ While MRLS is clearly associated with the hematogenous distribution of different bacterial species, it has not, to our knowledge, been possible to convincingly demonstrate a bacteremia associated with this condition. The SPSE portion of this hypothesis, suggesting that the invading

bacteria are carried in discrete quanta on small numbers of setal fragments, may offer an explanation for the lack of apparent evidence of bacteremia in natural and experimental MRLS mares. For example, a total of 10 setal fragments/day distributing in the blood of any single horse should yield about 1.5 LFL "hits"/day, and these LFL "hits" will be distributed through a relatively large volume (about 140 lbs) of fetoplacental tissue.

10.6/ This hypothesis is grounded in the well established physics and mechanics of the movement of barbed fragments through motile soft tissues, and the likelihood of bacterial contamination of such barbed fragments, as set forth in section 4. These bacteria then distribute throughout the horse as B, BE, or SPSE. We propose that this hypothesis accounts for all of the unique mathematical, epidemiological, clinical, pathological, and bacteriological characteristics of the five simultaneously occurring MRLS syndromes associated with exposure to ETC.

10.7/ If this setal hypothesis is correct, then similar exposure to mechanically and bacteriologically equivalent setae from other caterpillar species, or possibly from any other mechanically equivalent structure, may also have the potential to produce syndromes akin to MRLS. We note that while our experiment with Forest Tent Caterpillars failed to reproduce MRLS, a Gypsy Moth hairy caterpillar control in a colleague's experiment produced an abortion in one of four mares.

10.8/ Persons skilled in the art of experimental design will readily recognize appropriate experimental tests of this hypothesis. As a first step, it would be instructive to administer high doses of ETC to non-pregnant horses, followed by necropsy of the experimental animals. If the SPSE portion of this hypothesis is correct, then micropathological evidence of setal penetration in the intestinal tract and the intestinal portal circulation may be identified, as has recently been identified in pigs and rats, and apparently, recently, in a single horse [9]. Additionally, since the role of the setae is to carry bacteria, increased bacterial counts may be identified in tissues exposed to septic setal fragments. However, a concern is the small numbers and physical footprint of setal fragments, which may make identification of histopathological or microbiological changes in tissues distant from the intestinal tract difficult. As MRLS itself has shown, the fetoplacental unit of the late term mare is an exquisitely sensitive, if not the most sensitive, detector of SM spread.

The SPSE portion of this hypothesis is likely readily testable. Significant numbers of setal fragments, about the size of those observed in pig intestinal tracts, exposed to selected MRLS-related bacteria, (Table 1), suitably characterized or marked, genetically or otherwise, should induce MRLS after intravenous injection, and the bacteriology of the fetal membranes should reflect the specific bacterial additions to the setal fragments. A critical control would be intravenous injection of similar numbers of free bacteria. Injection of sterile setae should be much less effective than bacteria-coated setae, although what the simple mechanical effect of setae themselves might be on a pregnancy is unclear. Similarly, amniocentesis with introduction of these same bacteria should also rapidly induce MRLS type abortions, and it would be instructive to see whether not such introductions produced a classical MRLS micropathology, including the characteristic funisitis.

Table 1 Bacteriological findings from MRLS 2001 fetuses, as reported by Donahue et al.

Bacterium isolated	Number (%) of fetuses
	2001
Non-beta hemolytic streptococci	223 (51.5)
Actinobacilli	74 (17.1)
Actinobacilli and non-beta hemolytic streptococci	8 (1.8)
Escherichia coli	7 (1.6)
Pantoea agglomerans	4 (0.9)
Serratia marcescens	2 (0.5)
Aeromonas spp.	4 (0.9)
Enterobacter spp.	0
Acinetobacter spp.	4 (0.9)
Beta-hemolytic streptococci	2 (0.5)
Staphlococcus spp.	1 (0.2)
Other coliforms	4 (0.9)
Other bacteria	4 (0.9)
No significant bacteria	70 (16.6)
Overgrown by saprophytes	26 (6.2)
TOTALS	433 (100)

Adapted from and reproduced with permission from Table 1, Donahue et al, 2003.

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